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# Multi-Locus DNA Fingerprinting of Channel Catfish *Ictalurus punctatus*

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*Abstract.*—Similarities among multi-locus DNA fingerprints of five channel catfish *Ictalurus punctatus* strains and the ability to identify the strain of a fish based on its fingerprint pattern were investigated. Five restriction enzymes and 13 multi-locus DNA probes were screened to identify enzyme-probe combination useful for DNA fingerprinting channel catfish. Restriction enzymes *Hinf* I and *Dpn* II, in combination with probes (CAC)n, (CGC)n, (CTC)n, (ATCC)n, and (GATA)n, produced useful fingerprints (20–30 resolvable bands for each enzyme-probe combination). Thirty individuals (3 pools of 10 individuals each) from each of five channel catfish strains (albino, Mississippi normal, USDA-102, USDA-102 select, and USDA-103) were fingerprinted with all useful enzyme-probe combinations. Band sharing among samples was higher within strains than among strains and band sharing among strains was higher for strains whose breeding history indicated a high degree of relatedness. Individual fingerprints of 18 fish from each of the USDA-102 select and USDA-103 strains revealed no strain-specific bands, but several diagnostic bands (present at high frequencies in either USDA-102 select or USDA-103 strains and at a low frequencies in other strains) were identified. Band sharing at diagnostic bands was used to correctly identify fish as USDA-102 select or USDA-103 strains with 82% accuracy from fingerprints of 17 USDA-102 select strain fish, 18 USDA-103 strain fish, and 38 fish collected from three commercial farms.

Channel catfish *Ictalurus punctatus* culture is the largest aquaculture industry in the United States. More than 430 million pounds of catfish were processed in 1994 with an average price paid to producers of $0.77 per pound (USDA 1996). Development and commercial use of improved germplasm (e.g., faster growing, disease resistant) have dramatically increased production efficiency in many livestock species, and similar production increases should be possible through genetic improvement of catfish. Therefore, one of the mission objectives of the Catfish Genetics Research Unit (CGRU), USDA-ARS is to develop gentically improved catfish strains for release to the industry. As part of the CGRU germplasm development program, we are attempting to identify strain-specific molecular markers for strains scheduled for release. Strain-specific markers will be useful for maintaining strain integrity and providing proof of strain-type following release of fish to the industry.

Polymorphisms at isozyme loci can be used to distinguish among blue catfish *I. furcatus*, channel catfish, and their hybrids, but levels of polymorphism at these loci are too low within channel catfish to be useful for strain identification (Carmichael et al. 1992). Multi-locus DNA fingerprinting, a technique used to visualize restriction fragments at numerous, highly polymorphic loci, has been used to estimate relationships among populations of other fish species (Dahle 1994; Spruell et al. 1994; Naish et al. 1995) and may be useful for strain identification in catfish. The objectives of this research were to identify combinations of restriction enzymes and DNA probes useful for DNA fingerprinting channel catfish, to compare fingerprint patterns of five strains of channel catfish maintained at the CGRU, and to determine if strains can be identified by DNA fingerprint patterns.

## Materials and Methods

DNA isolation and fingerprint preparation were performed using techniques described for DNA fingerprinting of poultry (Dunnington et al. 1990). Genomic DNA was isolated from 50 μL of whole blood by phenol:chloroform extraction and ethanol

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precipitation, 10 μg samples of DNA were digested with 10–20 units of restriction enzyme, and digested DNA was electrophoresed in a 0.8% agarose gel in 1X TBE at 35 volts for 60–65 h. After electrophoresis, gels were incubated in 0.2 N HCL for 10 min, 1.5 M NaCl and 0.5 N NaOH two times for 15 min, and 1 M Tris and 1.5 M NaCl two times for 15 min (Maniatis et al. 1989). Following incubation, DNA was transferred to nylon membranes (MSI, Westboro, Massachussetts, USA) by capillary action in 10X SSC, and then membranes were baked at 65 C for 1 h. Membranes were hybridized with alkaline phosphatase conjugated DNA repeat probes and subjected to washes of increasing stringency using solutions and protocols provided by the probes' manufacturers. Membranes were exposed to X-ray film (Amersham, Arlington Heights, Illinois, USA) for 2–4 h at 37 C. Autoradiograms were scanned (Sharp JX-330 Scanner, Mahwah, New Jersey, USA) and Advanced Quantifier® 1-D software (BioImage, Ann Arbor, Michigan, USA) was used to identify bands, estimate band molecular weight, and calculate band sharing from the resulting images. Band sharing was calculated as:

$$2(Nab)/(Na + Nb),$$

where Nab = number of bands shared between samples a and b, Na = total number of bands in sample a, and Nb = total number of bands in sample b. Error tolerance (the molecular weight two bands could deviate and still be considered a match) was set at 1.5%.

Five restriction enzymes: *Alu* I, *Dpn* II, *Hae* III, *Hinf* I, *Rsa* I, (New England Biolabs, Beverly, Massachusetts, USA) and 13 probes: (CA)n, (GA)n, (ACG)n, (CAC)n, (CGC)n, (CTC)n, (ACAG)n, (ATCC)n, (AGAT)n, Alu/Sli, MV1 middle repeat (FMC BioProducts, Rockland, Maine, USA), 33.15 and 33.6 (Cellmark Diagnostics, Germantown, Maryland, USA) were tested to identify restriction enzyme-probe combinations useful for DNA fingerprinting channel catfish. The number of repeat units in the probes from FMC BioProducts is proprietary information.

Fingerprints were produced with DNA isolated from blood of individual fish and from blood pools (mixes of equal amounts of blood from 10 individuals). Three pooled samples (a total of 30 fish) from each of five CGRU catfish strains (albino, Mississippi normal, USDA-102, USDA-102 select, and USDA-103) were fingerprinted and band sharing was calculated within and among strains. Eighteen individuals from each of two strains being developed for release (USDA-102 select and USDA-103) were fingerprinted and within-strain band sharing was calculated. Fingerprints of individual and pooled samples were examined to identify banding patterns unique to USDA-102 select and to USDA-103 strains. Fingerprints of an additional 18 USDA-102 selects, 19 USDA-103s, and 38 fish from three commercial catfish farms were analyzed to determine whether fingerprint patterns could be used to correctly identify fish as either USDA-102 select or USDA-103 strains.

## Results

Useful fingerprints were produced by digesting DNA with *Dpn* II or *Hinf* I and hybridizing with (CAC)n, (CGC)n, (CTC)n, (ATCC)n, and (AGAT)n oligonucleotide probes. Probe hybridization and wash conditions are listed in Table 1. These enzyme-probe combinations typically produced 20–30 scoreable bands (size range 4–23 kb) per sample. The enzyme *Alu* I produced good quality fingerprints with the same five probes, but preliminary data indicated within-strain bandsharing with *Alu* I was lower than with *Dpn* II or *Hinf* I and, therefore, *Alu* I fingerprints did not appear to be useful for strain identification. The remaining restriction enzymes and probes were not useful for fingerprinting catfish due to the presence of too many bands and/or intense background signal [*Hae* III, (CA)n, (GA)n,

TABLE 1. *Hybridization temperature and stringency wash conditions for oligonucleotide probes used to DNA fingerprint channel catfish.*

| Probe | Hybridization temperature[a] | Stringency wash conditions[a] |
|---|---|---|
| CAC | 47 C | Wash 1, 2× for 10 min at 47 C<br>Wash 2, 2× for 10 min at 47 C |
| CGC | 47 C | Wash 1, 2× for 10 min at 47 C<br>Wash 2, 2× for 10 min at 47 C |
| CTC | 45 C | Wash 1, 2× for 10 min at 45 C |
| ATCC | 42 C | Wash 1, 2× for 10 min at 42 C |
| GATA | 42 C | Wash 1, 2× for 10 min at 42 C |

[a] Hybridization buffer and wash solutions are proprietary products provided by the probe supplier, FMC BioProducts, Rockland, Maine, USA.

33.15, and 33.6)] or few to no bands [(*Rsa* I, (ACAG)n, (ACG)n, Alu/Sli and MV1)].

Band sharing within strains was generally 30–40% higher than band sharing among strains (Table 2). Band sharing within strains was lowest for albinos (mean for all enzyme-probe combinations = 60%) and highest for USDA-102 selects (mean for all enzyme-probe combinations = 86%). The USDA-102 select strain had high band sharing (mean for all enzyme-probe combinations = 78%) with its founder population, USDA-102. A sample fingerprint of pooled DNA samples digested with *Dpn* II and probed with (CGC)n is shown in Fig. 1. Within-strain band sharing values for the various enzyme-probe combinations used to fingerprint individual fish ranged from 37–53% for USDA-102 selects and from 38–47% for USDA-103s. No strain-specific bands were found in USDA-102 selects or USDA-103s, but several enzyme-probe combinations revealed 2–4 bands present in more than 75% of individuals from each of these strains (Fig. 2). These bands appeared to be absent or at a low frequency in fingerprint patterns of pooled samples from other CGRU strains.

Band sharing greater than 50% at 3–4 diagnostic bands for each of the following enzyme-probe combinations: *Hinf* I-(ATAG)n, *Hinf* I-(ATCC)n, *Dpn* II-(ATAG)n, and *Dpn* II-(CAC)n, resulted in correct strain assignment for 83% of the fish tested. Four USDA-102 selects and three USDA-103 fish did not have sufficient band sharing at the diagnostic bands to be correctly assigned to their strain and six fish from commercial farms met the band sharing criteria to be incorrectly assigned as either USDA-102 select or USDA-103 strains.

## Discussion

DNA fingerprints were produced in channel catfish using two restriction enzymes (*Hinf* I and *Dpn* II) and five probes [(CAC)n, (CGC)n, (CTC)n, (ATCC)n and (ATAG)n]. Within-strain band sharing of individual USDA-102 select (37–53%) and USDA-103 catfish (38–47%) were similar to band sharing values reported in other fishes (Harris et al. 1991; Bosworth et al. 1994; Spruell et al. 1994). The high levels of band sharing among DNA pools within strains and between USDA-102 selects and their founder strain, USDA-102, indicated that fingerprint patterns were reflective of genetic similarity within and among strains. The higher within-strain bandsharing observed for pooled DNA samples compared to that observed for individual samples is expected because in pooled samples bands present at high frequencies in a population are more intense compared to those present at a low frequency, and therefore band sharing is biased upward in pooled samples (Spruell et al. 1994). The frequency of a band in a population does not effect its intensity in fingerprints of individuals.

Multi-locus fingerprinting also has been used to accurately estimate genetic similarities among tilapia strains (Naish et al. 1995), inbred poultry lines (Plotsky et al. 1995), and cattle (Mannen et al. 1993). The relatively low within-strain band sharing of albinos was surprising because it was thought that this strain was founded by a

TABLE 2. *Mean band sharing (%) within and among five channel catfish strains fingerprinted with 10 restriction enzyme-oligonucleotide probe combinations.*

| Strain | | Albino | | USDA-103 | | Mississippi Select | | USDA-102 | | USDA-102 Select | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Restriction enzyme | | | | | | | | | |
| | | *Dpn* II | *Hinf* I | *Dpn* II | *Hinf* I | *Dpn* II | *Hinf* I | *Dpn* II | *Hinf* I | *Dpn* II | *Hinf* I |
| | Probe | | | | | | | | | | |
| Albino | CACn | 68 | 61 | 39 | 41 | 62 | 47 | 36 | 41 | 47 | 35 |
| | CGCn | 74 | 71 | 41 | 45 | 50 | 55 | 41 | 41 | 46 | 49 |
| | CTCn | 53 | 58 | 43 | 39 | 52 | 44 | 42 | 30 | 44 | 34 |
| | ATCCn | 46 | 44 | 40 | 28 | 45 | 30 | 40 | 32 | 36 | 27 |
| | ATAGn | 58 | 69 | 46 | 52 | 51 | 59 | 46 | 48 | 41 | 50 |
| Mean[a] | | 60 | 61 | 42 | 41 | 52 | 47 | 41 | 38 | 43 | 39 |
| USDA-103 | CACn | | | 86 | 74 | 53 | 39 | 44 | 40 | 41 | 30 |
| | CGCn | | | 83 | 78 | 51 | 41 | 48 | 32 | 45 | 38 |
| | CTCn | | | 87 | 76 | 52 | 44 | 34 | 41 | 31 | 43 |
| | ATCCn | | | 73 | 81 | 41 | 29 | 50 | 36 | 43 | 42 |
| | ATAGn | | | 84 | 71 | 49 | 38 | 56 | 36 | 51 | 42 |
| Mean[a] | | | | 83 | 76 | 49 | 38 | 46 | 37 | 42 | 39 |
| Mississippi Select | CACn | | | | | 84 | 72 | 58 | 42 | 49 | 47 |
| | CGCn | | | | | 79 | 72 | 54 | 31 | 39 | 39 |
| | CTCn | | | | | 76 | 66 | 45 | 41 | 44 | 39 |
| | ATCCn | | | | | 68 | 80 | 58 | 38 | 55 | 42 |
| | ATAGn | | | | | 84 | 77 | 59 | 51 | 45 | 43 |
| Mean[a] | | | | | | 78 | 73 | 55 | 41 | 46 | 42 |
| USDA-102 | CACn | | | | | | | 96 | 77 | 72 | 71 |
| | CGCn | | | | | | | 81 | 82 | 82 | 74 |
| | CTCn | | | | | | | 92 | 87 | 88 | 79 |
| | ATCCn | | | | | | | 94 | 79 | 86 | 68 |
| | ATAGn | | | | | | | 98 | 80 | 81 | 75 |
| Mean[a] | | | | | | | | 92 | 81 | 82 | 73 |
| USDA-102 Select | CACn | | | | | | | | | 86 | 78 |
| | CGCn | | | | | | | | | 88 | 83 |
| | CTCn | | | | | | | | | 91 | 86 |
| | ATCCn | | | | | | | | | 89 | 75 |
| | ATAGn | | | | | | | | | 96 | 84 |
| Mean[a] | | | | | | | | | | 90 | 81 |

[a] Mean represents the mean band sharing between strains across the five oligonucleotide probes used with each restriction enzyme.

limited number of fish from a single strain. The apparent low genetic similarity in the albino strain may be the result of the founder population being a mix of fish from two or more strains.

Our data indicate that multi-locus fingerprinting may be useful for catfish strain identification, but the accuracy of assigning fish to a strain must be improved. Culling from a strain fish that did not have the strain's characteristic banding pattern could be used to improve accuracy of identification by fingerprinting. Development and use of locus-specific probes for diagnostic bands could also improve the accuracy of strain assignment by eliminating band sharing due to co-migration of non-allelic fragments (O'Reilly and Wright 1995).

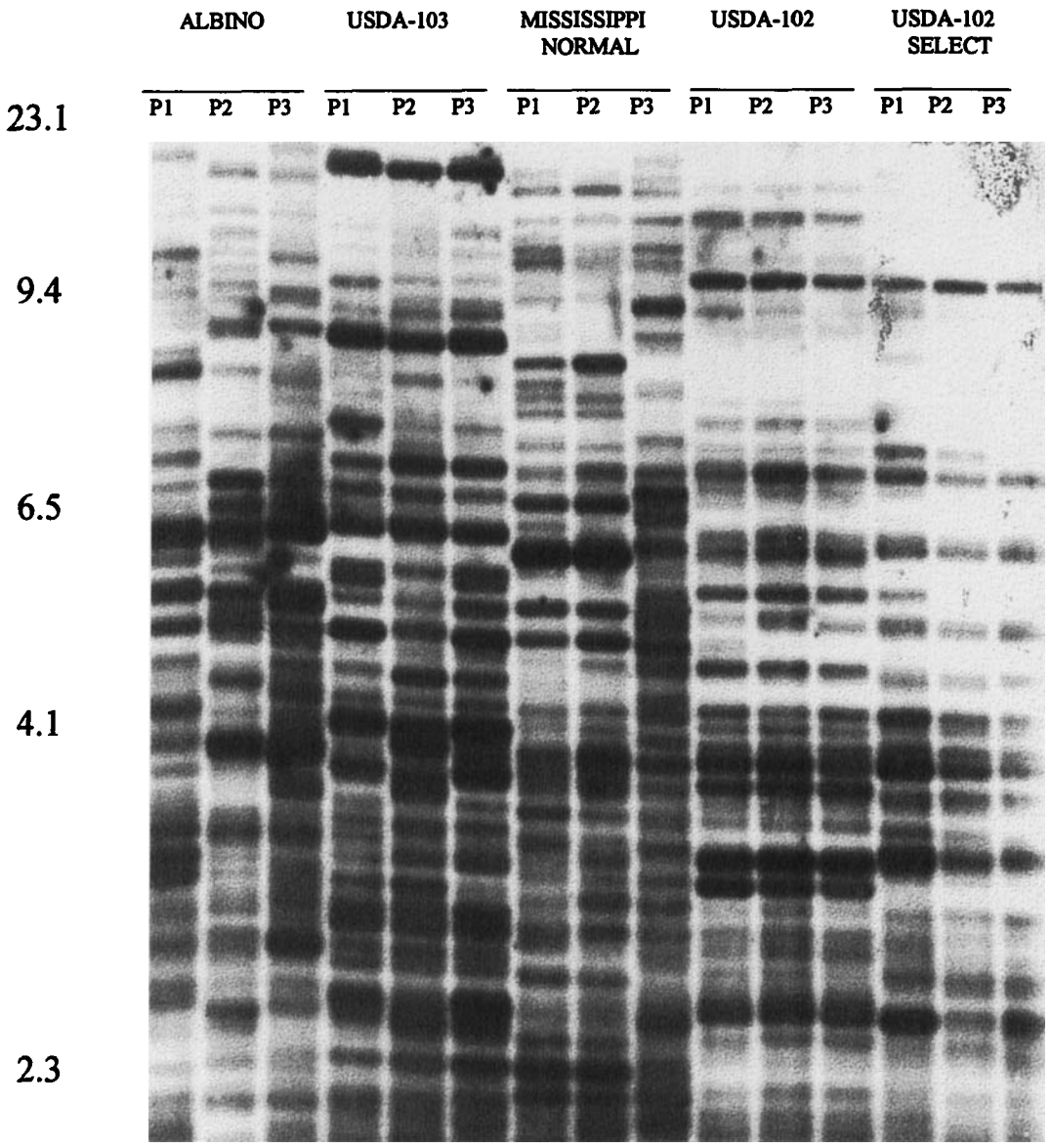


FIGURE 1. *DNA fingerprint of albino, USDA-103, Mississippi normal, USDA-102, and USDA-102 select strains of channel catfish. Each lane contains DNA isolated from blood pooled from ten fish, three pools (P1, P2, and P3) per strain. DNA was digested with Dpn II and probed with (CGC)n, approximate molecular weights in kb are listed in the left margin.*

Multi-locus DNA fingerprinting with non-radioactive probes can be conducted in labs with minimal equipment and allows simultaneous screening of numerous polymorphic loci. However, disadvantages of multi-locus fingerprinting include the time needed to produce results (5–6 d), amount of DNA required (5–10 μg per sample), and variation in banding patterns due to slight differences in transfer efficiency or hybridization and wash conditions (O'Reilly and Wright 1995). Therefore, we are also examining the ability to identify catfish strains based on polymorphisms at microsatellite

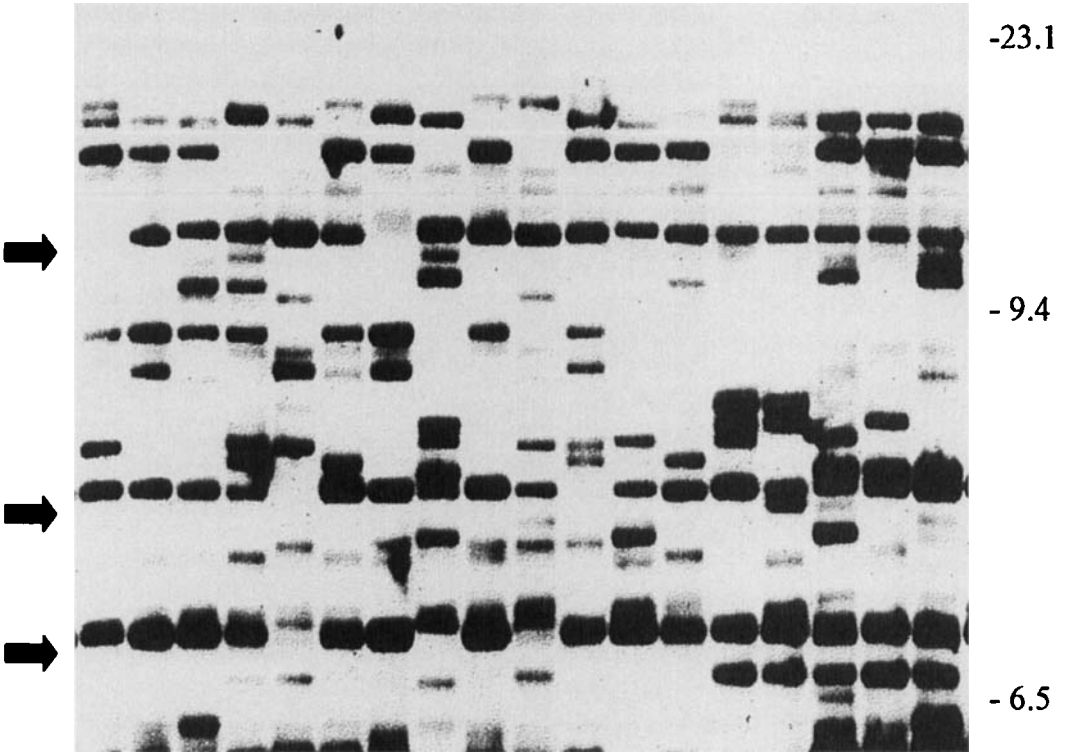


FIGURE 2. *DNA fingerprint produced from individual USDA-103 strain channel catfish with the restriction enzyme* Dpn *II and the oligonucleotide probe (ATAG)n. Arrows designate three diagnostic bands for the USDA-103 strain and molecular weights (kb) are listed in the right margin.*

loci. Although initial costs for developing primers for a large number of microsatellite loci are high. PCR amplification of microsatellites requires small amounts of DNA and will allow rapid and consistent genotyping of large numbers of fish.

Regardless of the techniques used to visualize polymorphism, the use of highly variable DNA markers appears to have potential for strain identification in catfish. Use of strain-specific markers will allow producers who propagate and distribute released strains to maintain genetic integrity of their stocks and provide proof of strain integrity to individuals interested in purchasing a particular strain.

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